PrimaPure™

Human Marrow Stromal Cells (HMSC)

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Description/Content	Amount
SH49205	Cryopreserved HMSC	>500,000 cells
SH49205K	HMSC Complete System	1 Kit*
*Each kit contains an ampoule of cryopreserved HMSC (SH49205F), 500 ml of Human Marrow Stromal Cell Growth Medium (SMH419500), and a Subculture Reagent Kit (PR090100K).		

Related Products	Catalog #
Human Marrow Stromal Cell Growth Medium, 500 ml	SMH419500
Subculture Reagent Kit, including100 ml each of HBSS, Trpsin/EDTA, and Trpsin Neutralizing Solution	PR090100K
Osteoblast Differentiation Medium, 500 ml	SMH417D250
Adipocyte Differentiation Medium, 500 ml	SMH811D250

Storage:	Store cryopreserved vials in liquid nitrogen immediately upon arrival. Store the growth medium at 4°C in the dark immediately upon arrival. Store the Subculture Reagent Kit at -20°C upon arrival and store the reagents at 4°C upon thawing.

INTRODUCTION

Human Marrow Stromal Cells (HMSC) are isolated from fetal human bone marrow. They are cryopreserved at second passage and can be cultured and propagated 10 population doublings. In addition to supporting hematopoietic cells[1], marrow stromal cells can be induced to differentiate into cells of different connective tissue lineage, such as bone, cartilage, and fat[2,3,4]. The potential of HMSCs to maintain multipotency and proliferate extensively *in vitro* provides new avenues for cell-based therapy in the restoration of damaged or diseased tissue[5,6]. Recent reports indicate that HMSCs are capable of cell fates crossing germ layer boundaries. In addition to differentiating into multi-lineages of the mesoderm, these cells can also differentiate into neurons[7] of ectodermal origin and hepatocyte-like cells[8] of endodermal origin.

MATERIALS AND METHODS

I. Preparation for Culturing

1. Make sure your Class II Biological Safety Cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips, and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
   c. Handle all cell culture work in a sterile hood.

II. Culturing MNSC

A. PREPARING CELL CULTURE FLASKS FOR CULTURING HMSC

1. Take the Human Marrow Stromal Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 15 ml of Human Marrow Stromal Cell Growth Medium* into a T-75 flask.

* Keep the medium to surface area ratio at 1 ml per 5 cm^2.
For example, 5-7.5 ml for a T-25 flask or a 60 mm tissue culture dish. 15-20 ml for a T-75 flask or a 100 mm tissue culture dish.

B. THAWING AND PLATING MNSC

1. Remove the cryopreserved vial of HMSC from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 1 minute.
4. Take the vial out of the water bath and wipe dry.
5. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
6. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
7. Resuspend the cells in the vial by gently pipetting the cells 5 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.
8. Pipette the cell suspension (1 ml) from the vial into the T-75 flask containing 15 ml of Human Marrow Stromal Cell Growth Medium.
9. Cap the flask and rock gently to evenly distribute the cells.
10. Place the T-75 flask in a 37oC, 5% CO_2 humidified incubator. Loosen the cap to allow gas exchange. For best results, do not disturb the culture for 24 hours after inoculation.
11. Change to fresh Human Marrow Stromal Cell Growth Medium after 24 hours or overnight to remove all traces of DMSO.
12. Change Human Marrow Stromal Cell Growth Medium every other day until the cells reach 60% confluent.
13. Double the Human Marrow Stromal Cell Growth Medium volume when the culture is >60% confluent or for weekend feedings.
14. Subculture the cells when the HMSC culture reaches 85-95% confluent.

III. Subculturing HMSC

A. PREPARING SUBCULTURE REAGENTS

1. Remove the Subculture Reagent Kit from the -20°C freezer and thaw overnight in a refrigerator.
2. Make sure all the subculture reagents are thawed. Swirl each bottle gently several times to form homogeneous solutions.
3. Store all the subculture reagents at 4°C for future use. The activity of Trypsin/EDTA Solution will be stable for 2 weeks when stored at 4°C.
4. Aliquot Trypsin/EDTA solution and store the unused portion at -20°C if only portion of the Trypsin/EDTA is needed.

B. PREPARING CULTURE FLASK

1. Take the Human Marrow Stromal Cell Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 35 ml of Human Marrow Stromal Cell Growth Medium to a T-175 flask (to be used in Section III C Step 15).

C. SUBCULTURING HMSC

Trypsinize Cells at Room Temperature. Do Not Warm Any Reagents to 37°C.

1. Remove the medium from culture flasks by aspiration.
2. Wash the monolayer of cells with HBSS and remove the solution by aspiration.
3. Pipette 5 ml of Trypsin/EDTA Solution into the T-75 flask. Rock the flask gently to ensure the solution covers all the cells.
4. Remove 4 ml of the solution immediately.
5. Re-cap the flask tightly and monitor the trypsinization progress at room temperature under an inverted microscope. It usually takes about 2 to 5 minutes for the cells to become rounded. The cells may not be completely round during trypsinization and some cells may maintain some processes even though they are loosened from the culture surface.
6. Release the rounded cells from the culture surface by hitting the side of the flask against your palm until most of the cells are detached.
7. Pipette 5 ml of Trypsin Neutralizing Solution to the flask to inhibit further tryptic activity.
8. Transfer the cell suspension from the flask to a 50 ml sterile conical tube.
9. Rinse the flask with an additional 5 ml of Trypsin Neutralizing Solution and transfer the solution into the same conical tube.
10. Examine the T-75 flask under a microscope. If there are >20% cells left in the flask, repeat Steps 2-9.
11. Centrifuge the conical tube at 220 x g for 5 minutes to pellet the cells.
12. Aspirate the supernatant from the tube without disturbing the cell pellet.
13. Flick the tip of the conical tube with your finger to loosen the cell pellet.
14. Resuspend the cells in 5 ml of Human Marrow Stromal Cell Growth Medium by gently pipetting the cells to break up the clumps.
15. Count the cells with a hemocytometer or cell counter. Inoculate at 10,000 cells per cm^2 for rapid growth, or at 5,000 cells per cm^2 for regular subculturing.

IV. Differentiating MNSC

A. SEEDING HMSC FOR DIFFERENTIATING INTO OSTEOBLASTS

1. Seed HMSC in the desired format at 10,000 per cm^2.
2. Place the cells 37°C, 5% CO_2 humidified incubator.
3. Change to Osteoblast Differentiation Medium (sold separately) the next day by removing the growth medium from culture tissue ware by aspiration and adding the appropriate volume of Osteoblast Differentiation Medium. Do not allow cells to dry during medium changes.
4. Incubate cell in a 37°C, 5% CO_2 humidified incubator.
5. Change to fresh Osteoblast Differentiation Medium every three (3) days.
6. Extracellular matrix will be mineralized in 30 days.

B. SEEDING HMSC FOR DIFFERENTIATING INTO ADIPOCYTES

1. Seed the HMSC at 40,000 cells/cm^2 in the desired format.
2. Place the cells in a 37°C, 5% CO_2 humidified incubator.
3. Change to Adipocyte Differentiation Medium (sold separately) after three days by removing growth medium from culture tissue ware by aspiration and adding the appropriate volume of Adipocyte Differentiation Medium. Do not allow cells to dry during medium changes.
4. Incubate cell in a 37°C, 5% CO_2 humidified incubator.
5. Change to fresh Adipocyte Differentiation Medium every three (3) days.
6. Cells are differentiated into adipocytes with large lipid droplets in 3-4 weeks.

REFERENCES

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